Researchers improve design of genetic on-off switches

April 7 2005

Researchers at the University of Illinois at Urbana-Champaign have set a new standard in the design and engineering of nuclear hormone receptor-based genetic on-off switches, without causing new problems or aggravating existing ones.

The new technique, published online ahead of regular publication by the Proceedings of the National Academy of Sciences, combines the advantages of directed evolution and computationally driven rational design, said Huimin Zhao, a professor in the department of chemical and biomolecular engineering and member of the Institute for Genomic Biology at Illinois.

Zhao's team, using yeast and mammalian cells, altered the specificity of human estrogen receptor alpha by 100 million times so it would bind preferentially to a non-toxic synthetic molecule (4,4'-dihydroxybenzil) over the natural estrogen 17-beta-estradiol.

Such selectivity moves researchers closer to designing synthetic molecules that will attach to only targeted receptors to activate or deactivate desired gene expression in living systems, which could lead to advances in such applications as gene therapy, metabolic engineering, functional genomics, enzyme engineering and animal disease model studies.

Many previous attempts, using a variety of molecular methods, have involved time-consuming approaches that have resulted in unintended

activity when non-targeted receptors have responded to the new molecules.

“I’m not saying that we have solved the problem, but we have shown that our approach can be very efficient and done successfully,” said Zhao, also an affiliate in the chemistry and bioengineering departments and member of the Center for Biophysics and Computational Biology. “We were able to alter the ligand (molecule) selectively by 10 to the 8th in mammalian cells. No one has had this high level of success.”

The Illinois approach, Zhao said, is more general, quicker to accomplish and more accurate than a scientifically hailed combinational approach published in PNAS last October by researchers at the Georgia Institute of Technology. In their paper, the Georgia scientists used random mutagenesis and chemical complementation to develop a yeast-based system that made a retinoid X receptor, a nuclear hormone receptor, recognize and bind to a synthetic molecule.

The protein-engineering approach used by Zhao’s team used directed evolution, which mimics natural evolution in a test tube, to force rapid evolution of human estrogen receptor with new ligand specificity. This process is done mainly through stepwise, site-saturation mutagenesis and high throughput screening. The sites of human estrogen receptor chosen for saturation mutagenesis were identified through rational design, which involves computational modeling and biochemical and genetic studies to predict the interactions between the receptor and the ligand and the myriad molecular interactions that take place to drive gene expression. The engineered genetic changes subsequently make the receptor highly sensitive to the synthetic molecule that is introduced.

“We envision that the described technology could provide a powerful, broadly applicable tool for engineering receptors/enzymes with improved or novel ligand/substrate specificity,” Zhao said.

Co-authors with Zhao were Karuppiah Chockalingam and Zhilei Chen, both doctoral students in chemical and biomolecular engineering, and John A. Katzenellenbogen, a Swanlund Endowed Chair in chemistry and affiliate of the Beckman Institute of Advanced Science and Technology at Illinois.

A patent is being sought for the protein-engineering technology and gene switch.

A National Science Foundation Faculty Early Career Development Award grant to Zhao funded the research.

Source: University of Illinois at Urbana-Champaign